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MORPHOLOGICAL DIVERSITY OF PHYTOLITH TYPES IN SOME CHLORIDOID GRASSES OF PUNJAB

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ABSTRACT

The deposition of phytoliths in some chloridoid grasses have been investigated. Morphology, size and distribution patterns of phytoliths were examined in the adaxial surface of leaf blades. Dumb-bell shape was found to be the most common, usually arranged in two or three rows followed by trapezoid and dendritic phytolith types. Lanceolate, clavate and cross were the less frequent types.

KEYWORDS: Phytoliths, Grasses, Dumb, Bell, Morphology, Chloridoid

INTRODUCTION

Phytoliths (Greek, *phyto*= plant, *lithos*= stones) or plant stones are amorphous forms of silicon dioxide (Sio_2) (known as "opal") found in many plants (Mazumdar, 2009). The term "phytolith" refers only to microscopically recognizable shapes, not amorphous pieces or traces of silica detectable only by microchemical methods that would not be recognized as discrete types (Piperno, 2006). Monosilicic acid ($Si(OH)_4$), in the soil, created from the weathering of rocks and the dissolution of biologically deposited silica is taken up by plants. Following uptake the acid is transported to various plant organs, where, in many taxa, some of it polymerizes to form solid silica deposits at specific intracellular and extracellular locations. As the acid solidifies, the resulting phytoliths take on the shape of the cell or tissue in which they form. Phytoliths have been found to be advantageous for the growth and development of various plants (Jarvis, 1987; Matoh et al., 1991). It assist plants to overcome various biotic and abiotic stresses (Epstein, 1999; Ma and Yamaji, 2006).The existence of phytoliths in the plant tissues has structural and protective role against fungi, insects, and herbivores. Silica bodies are abundant and morphologically distinct as well as much durable in soil for thousand even millions of the year after death and decay of the plants (Rovner, 1971; Piperno, 1988) The structural hierarchy and durability of this element in the form of phytoliths in different parts of the plant (Metcalfe, 1960; Chauhan et al., 2009) as well as in soil has proven the possibilities to solve the archaeological problems and to identify the crop plants which were grown in our past (Baker, 1960; Twiss, 1987). Majority of archaeologists and palaeoecologists have been using this kind of study for the reconstruction of palaeoenvironments, palaeoclimate and the presence of forested vegetation, soil horizons as well as past plant-people relationship (Mulholland, 1989; Mulholland and Rapp, 1992). The mapping of Silica bodies (phytoliths) from different ecological zones is very much beneficial to analyze the probable reasons of biodiversity depletion and to understand the relationship between human and land (Pearsall, 2000). The present study examines the occurrence, morphology and diversity of phytoliths in the leaves of some Chloridoid grasses of Punjab.

MATERIALS AND METHODS

Area of Study

The grass species in the present study were collected from the Punjab plains in the North-Western Himalayan region. Physiographically, it is a plain region with an average elevation of 234.14 m asl. Mean annual rainfall in the region

is about 90-115 cm which is received mostly during the rainy season. Annual mean maximum and mean minimum temperatures are 31.3^0 C and 13.25^0 C respectively. Whole plant specimens were collected at flowering stage, cut to size and preserved in 70% ethanol at 4^oC.

Phytolith Analysis

Phytoliths were located in epidermal cells by the clearing solution method of Krishnan (2000). Leaf segments were washed and immersed in a clearing solution of Lactic acid and Chloral hydrate (3:1) and kept at 70^0 C for 2 days. Adaxial surface of cleared segments were mounted in fresh solution and observed under light microscope (40X).

The method of Carnelli et al. (2001) was followed for dry ashing of the material. The material was rinsed and cut into small pieces and heated to ashes in porcelein crucibles in a Muffle Furnace maintained at 470 ^{0}C for 48 hours. The crucibles were taken out, cooled and the contents transferred to test tubes. Sufficient amount of Hydrogen peroxide (30%) was added to submerge the contents and the test tubes were kept at 80 ^{0}C for 1 hour. The test tubes were taken out from the incubator; the mixture was decanted and rinsed twice with distilled water. Hydrochloric acid (10%) was added to the pellet and incubated again for 1 hour. Thereafter, the mixture was washed with distilled water and centrifuged at 7500 rpm for 10 minutes. The supernatant was decanted and the pallet was washed twice with distilled water. The centrifugation process was repeated four times till the pallet was clear. Finally, the pallet was dried for 24 hours at 60 ^{0} C to a powder form. In this form, the material was taken in small bits and mounted on glass slides in DPX for optical microscopy. Olympus Micro Image Projection System (MIPS-USB 0262) was used for microphotography. Photographs were taken at a uniform magnification for ease of comparison. Phytoliths were classified into types and subtypes according to the International Code of Phytolith Nomenclature (Madella *et al*., 2005).

Morphometry and Statistical Analysis

Morphometric measurement of various types of phytoliths was done with Image J software (version 1.46r.). It is userfriendly software that allows measurements of overall size and other dimensional aspects of microscopic objects from their microphotographs. In the present study, twenty phytoliths of each type from different grass species in the sample were photographed with the help of a Micro Image Projection System (Olympus) and stored in separate files for various species. Thereafter, dimensions of phytoliths were recorded with the help of the Image J software. After loading the software, images of phytoliths were retrieved into the current file (RAM) of the computer. The software records dimensions as the cursor is dragged along the dimensions (length and width) in the images of the objects photographed. The software not only records length and width but also calculates other morphometric parameters *viz.,* aspect ratio, surface area, roundedness and solidity. In the present paper, we have included data on the length and width (µm) only. Mean and standard error was calculated with the help of PAST software (Paleontological Statistics software).

Scanning Electron Microscopy

Details of shape and surface features of phytoliths were studied with the help of Scanning Electron Microscopy. Dry ash was spread uniformly over the stubs with the help of double-sided adhesive tape. The stubs were put under a stereoscope for uniform spreading of the ash. Silver paint was applied on the edges of the stub. The samples were dried at 40^0C overnight. Next day, stubs were coated with graphite using a vacuum evaporator (JEOL-JEE-4X). They were subsequently coated with gold by a sputter coater (POLARON) and imaged under SEM (ASID) at an accelerating voltage of 40KV.

RESULTS

Morphological diversity of phytoliths in the leaves of 9 Chloridoid grass species exhibited considerable variation with respect to shape, size, distribution patterns and position on leaves (Table 1). Table 2 lists the representative grass taxa of different phytolith types, their common names, ecology and distribution. In grass leaves some of the coastal cells cells (long silica cells) and some of the intercostals cells (short silica cells) usually forming part of silico-suberose couples normally acquire silica at an early stage and in a regular manner. The bodies vary in shape from, straight or smooth-sided to lobed, with two or three lobes on each edge. The various shapes of phytoliths were observed, including dumb-bell (bilobate) dendric, trapezoid, rondel, rectangular, saddle, smooth elongate, oval, lenceolate, cross and clavate shapes. Since the morphology of phytolith is most consistant and a diagnostic feature, futher results are described in the sequel with respect to the shape.

Dumb-Bell or Bilobate Shaped Phytoliths: Usually dumb-bell shaped phytoliths are arranged in rows on the adaxial surface of the leaves on both sides of the veins (Figure 1p). They are also present in the form of sheets (Figure 2e). Dumb-bell shaped phytoliths show considerable morphological diversity with respect to the thickness of the shank, size of lobes and the depression (notch) on the lobes (Figures 1l-1r)). *Desmostachya bipinnata* (L.) Stapf. bears thick shank and clear cut lobes as compared to the other members (Figures 1l, 1m). They were reported in all the members of the present sample except *Tragus biflorus* Schult.

Dendritic Shaped Phytoliths: These types were reported in almost all members except *Desmostachya bipinnata* (L.) Stapf and *Leptochloa chinensis* (L.) Nees. Dendritic phytolith types also showed considerable diversity with mean size (length) reaching up to 39.90µm. They show wavy or bristle like extensions on their outer surface (Figures 1a-1e). These bristles may be due to deposition of phytoliths in the intercellular spaces. Scanning electron micrographs clearly reveal the bristle like extensions in *Dactyloctenium aegyptium* (L.) Willd. (Figure 2b).

Trapezoid Phytoliths: These phytolith types were also variously shaped (Figsure 1u, 1v, 2f). *Neyraudia arundinacea* (L.) Henrard shows wavy trapezoids (Figsure 1u, 1v) but, in other members of the present sample trapezoids are variously shaped.

Rondel (Circular to Oval Phytoliths) Shaped Phytoliths: These phytoliths were found randomly distributed along with the saddle types (Figure 1w).

Rectangular Phytolith Types: These are derived from epidermal long cells, trichome cells and bulliform cells of grasses. Their surface is almost smooth (Figures 1s, 1t).

Saddle Shaped Phytoliths: These phytolith types were reported in *Dactyloctenium aegyptium* (L.) and *Sporobolus diandrus* (Retz.) P. Beauv. *Dactyloctenium aegyptium* (L.) has both long and short saddles while *Sporobolus diandrus* (Retz.) P. Beauv has only short saddles.

Smooth Elongate Phytolith Types: These are rod like phytoliths which are also derived from epidermal long cells, trichome cells and bulliform cells of grasses. Their surface is highly smooth and reaches up to the length of 129.67 µm in *Leptochloa panicea* (Retz) Ohwi. (Table 1) (Figures 1i-1k, 2a, 2c). They are also arranged in bundles in *Leptochloa panicea* (Retz) Ohwi.

The other less frequent types were oval (Figures 2h, 2i), clavate (2j), cross (Figure 1x) and lenceolate (Figure 1y) phytolith types.

DISCUSSIONS

Study of intact and isolated phytolith types adds significantly to our knowledge of their morphological diversity and distribution patterns. During our study 11 types of phytoliths were observed with huge morphological diversity in each type (Plate I and plate II). These shapes were reported by earlier workers in different grasses (Blackman, 1971; Sangster, 1978; Bozarth, 1992; Jattisha and Sabu, 2012). Dumb-bell shaped phytoliths were found to be the most common. It was found in the 8 out of 9 taxa studied. Dendritic and trapezoid types were found in 7 out of 9 taxa. The less common types found were oval, clavate, cross and laceolate shaped phytoliths. Dumbell shaped phytoliths types showed huge morphological diversity with respect to the thickness of shank, size of lobes and the depression (notch) on the lobes. Similarly, dendritic and trapezoid types also showed morphological as well as morphometric diversity. Further, scanning electron microscopy has also brought out the shape and size of bristles (entensions) on the surface of dendritic phytolith types. The variation in these phytolith types could have a great diagnostic potential. Many studies have been conducted to understand these variations. Several intrinsic and extrinsic factors seem to control these variations *viz.,* stage of plant maturity (Hodson, Sangester and Parry, 1985), intraspecific variation within plant taxa (Piperno, 1984), the rate of leaf transpiration (Rosen, 1983) , the tissue type (Blackman, 1971) and the amount of soluble silica in ground water. Hence, there is an urgent need to understand and evaluate the effect of these variables before one could utilize the full potential of phytoliths as diagnostic markers in plant systematics.

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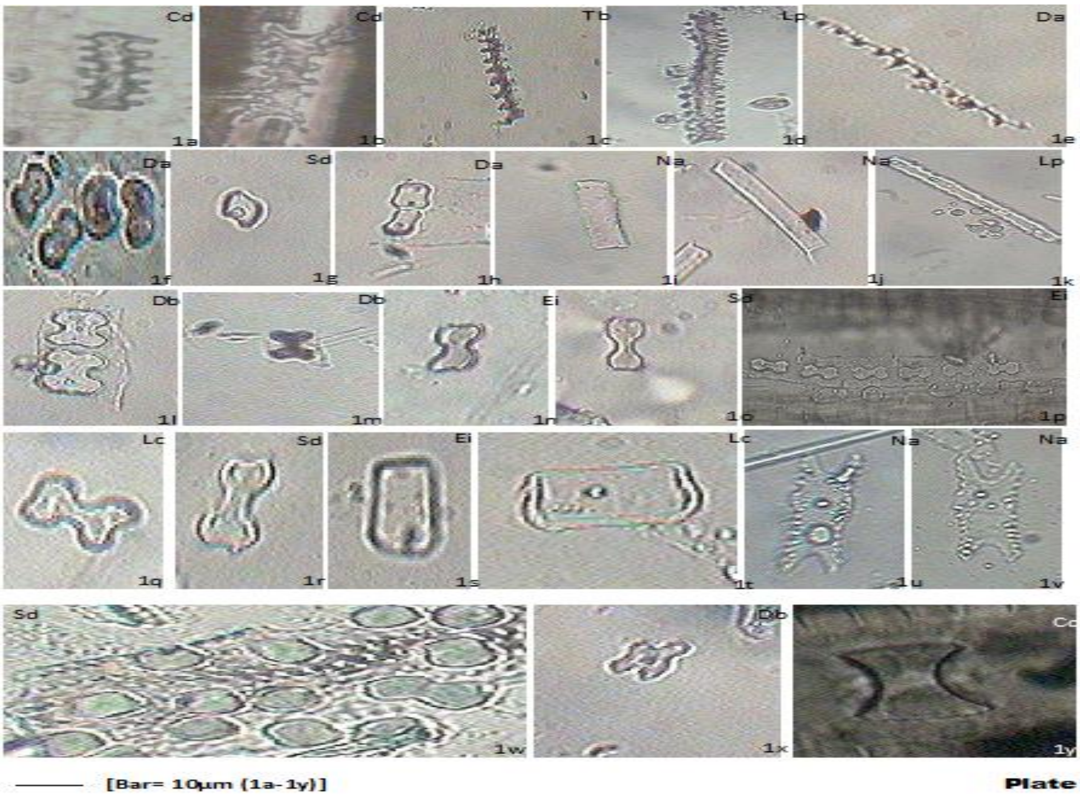
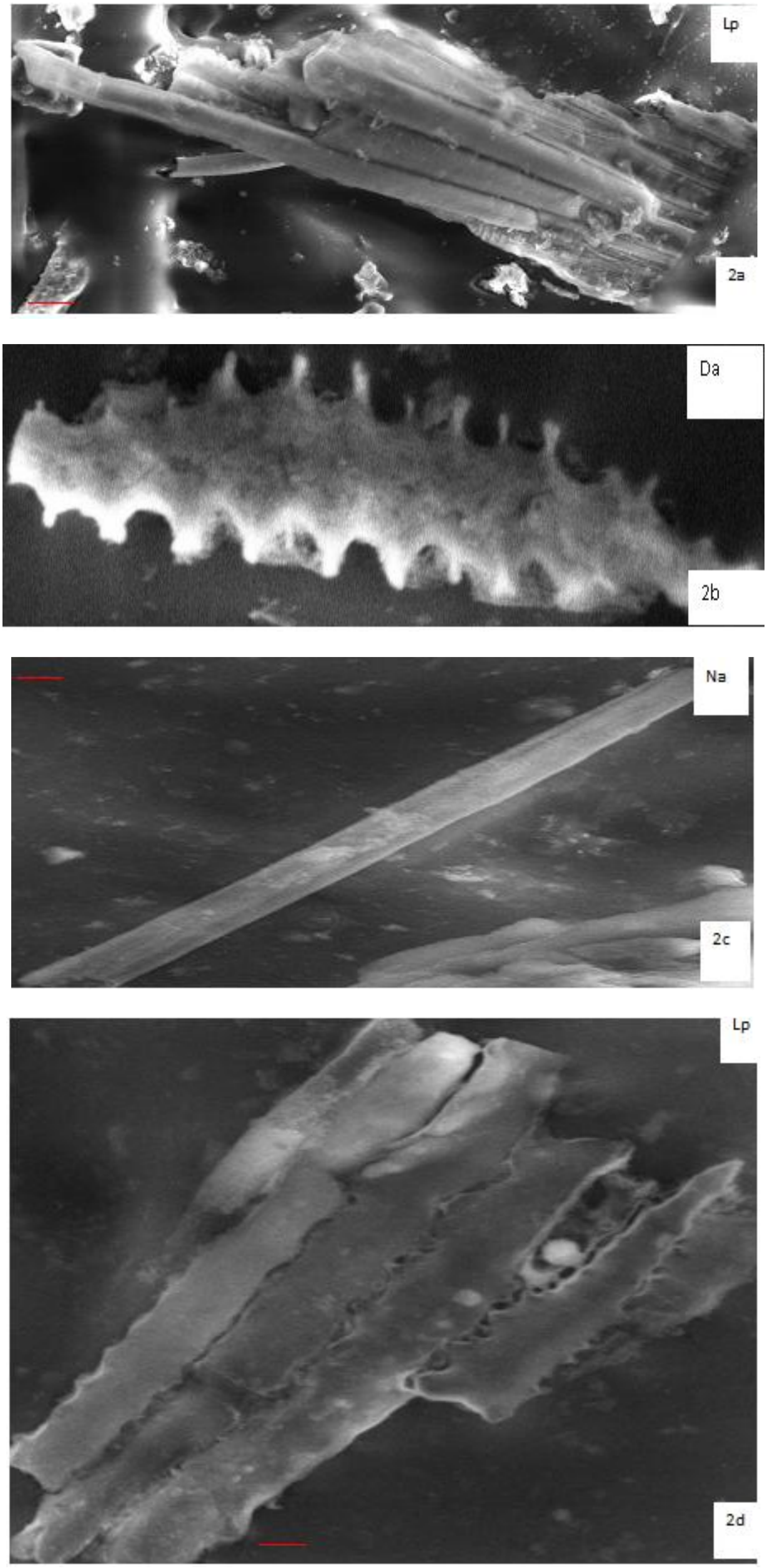


Figure 1: Light Microscope Images (Figures. 1a-1y): Dendritic (1a-e), Saddle (1f-h) Smooth Elongate (1i-ik), Dumbell or Bilobate (1l-1r), Rectangular (1s, t) Trapezoid (1u, v), Saddles & Rondels (1w), Cross (1x), Lenceolate (1y)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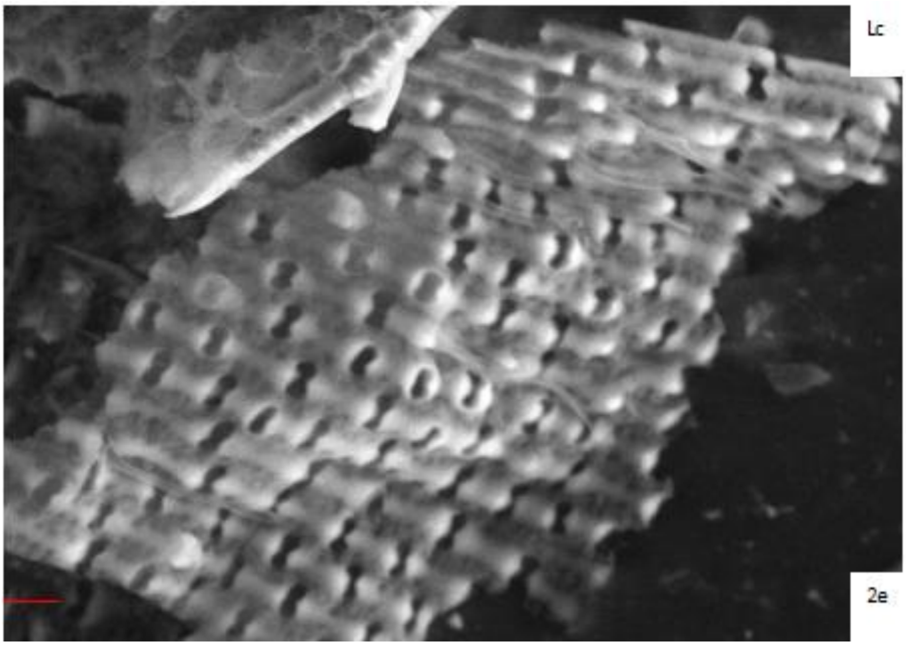
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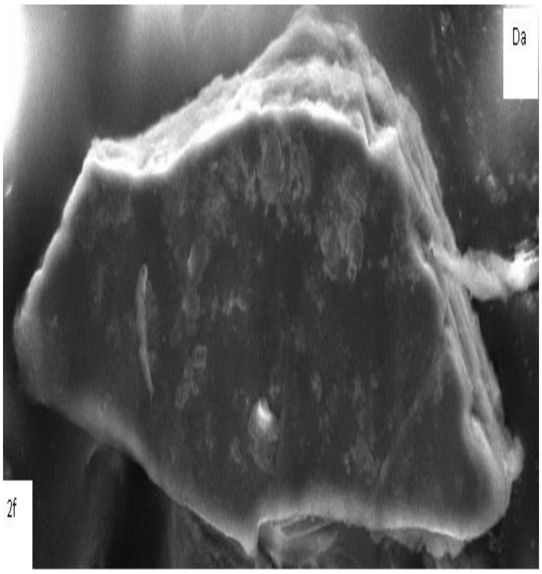
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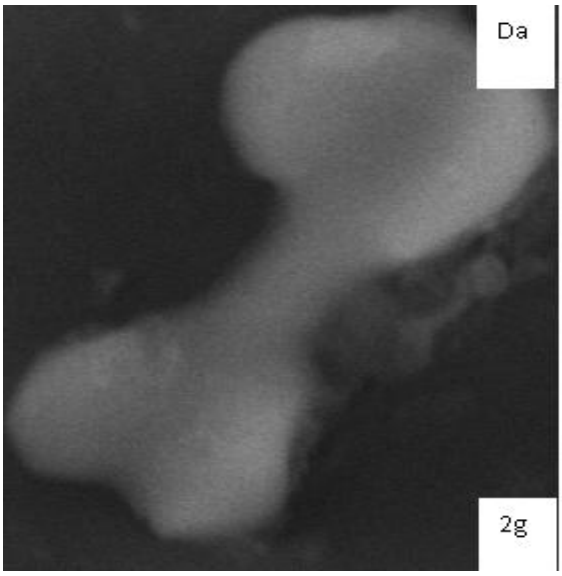
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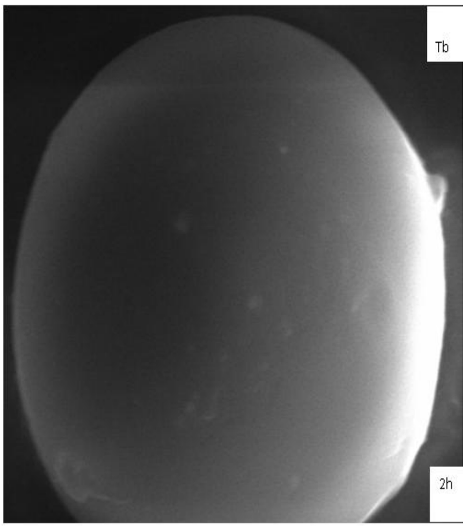
Tb
2h

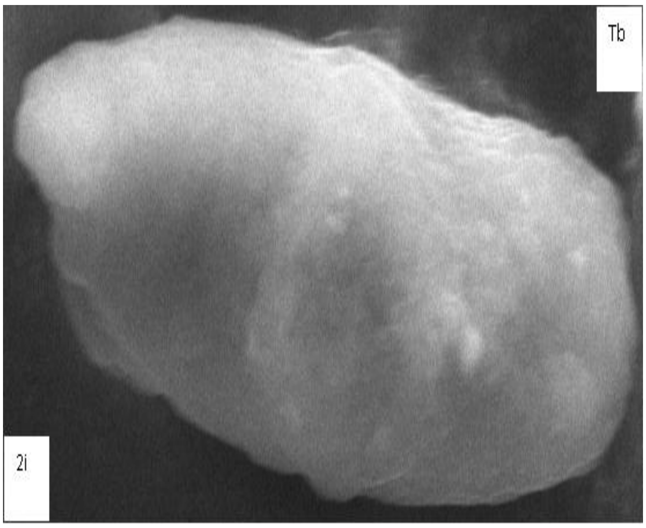
Tb
2i

Figure 2: Scanning Electron Micrographs (Figures 2a-2i): Smooth Elongate [2a (Bar=20µm], 2c (10 µm)], Dendritic [2b (10 µm), 2d (10 µm)], Bilobate [2e (10 µm), 2g (2 µm)], Trapezoid [2f (40 µm)], Oval [2h (2 µm), 2i (10 µm)], Clavate [2j (10 µm)]

Table 1: **Data on Presence/Absence and Size Dimensions of Phytoliths in Chloridoid Grasses of Present Sample**

Name of Species	**Phytolith Morphotypes and their Size Dimensions (µm)**												
	Abv	**Size Dimensions**	**BL**	**CL**	**CR**	**DT**	**LCN**	**OVL**	**RD**	**RT**	**SD**	**SmE**	**TZ**
Subfamily: Chloridoideae													
Tribe: Cynodonteae													
*Cynodon dactylon***(L.) Pers**	Cd	Length Width	*11.3 ±0.59 #4.9± 0.17	–	–	29.6± 2.025 .6±0. 26	22.1± 1.386 .8±0. 48	–	28± 1.06 5.3± 0.3	–	–	–	29±0. 965.5 ±0.22
Tragus biflorus **Schult.**	Tb	Length Width	–	–	–	39.90 ±2.86 8.63± 0.82	–	9.03±0 .847.4 4±0.42	–	36.98 ±2.17 14.63 ±1.64	–	–	51.62 ±4.18 31.70 ±2.41
\Tribe: Eragrostideae			–	–	–	–	–	–	–	–	–	–	–
*Dactyloctenium aegyptium***(L.) Willd.**	Da	Length Width	20.3± 1.04.7 ±0.15	–	–	39±0. 665.3 ±0.30	–	–	28± 1.06 5.3± 0.3	–	7.1± 0.64 6.1± 0.43	–	–
*Desmostachya bipinnata***(L.) Stapf**	Db	Length Width	17.5± 0.769. 1±0.2 7	–	09±0.3 96.9±0 .23	–	–	–	6.9± 0.48 4.3± 0.21	–	–	–	–
Eleusine indica **(L.) Gaertn.**	Ei	Length Width	16.7± 0.786 ±0.25	–	–	29.2± 2.885 .7.3± 0.15	–	–	12.1 ±1.2 36.3 ±0.3 6	27.9± 1.707 .3±0. 33	–	–	18.9± 1.508 .9±0. 27
*Leptochloa chinensis***(L.) Nees**	Lc	Length Width	15.2± 1.315. 4±0.2 2	–	–	–	–	–	–	26.3± 2.151 1.4±0 .4	–	–	15.7± 1.071 0.0±1 .32
Leptochloa panacea ***(Retz)Ohwi.***	Lp	Length Width	16.27 ±1.31 6.43± 0.67	–	–	37.81 ±2.85 8.25± 0.56	–	–	–	95.43 ±7.77 38.52 ±6.94	–	129. 67± 14.3 27.8 8±0. 74	59.24 ±5.41 32±2. 66

Table 1: Contd.,

Neyraudia arundinacea (**L.**) **Henrard**	Na	Length Width	10.1±0.925.3±0.67	37.4±1.246.4±0.22	–	33.7±2.36.5±0.22	–	–	–	–	–	40.2±2.28.4±0.30	24.2±1.3816.4±0.96
Sporobolus diandrus (**Retz.**) **P. Beauv.**	Sd	Length Width	22.4±1.826.9±0.15	–	–	26.2±1.515.7±0.15	–	108±0340±0.5	8.3±0.54.7±0.26	–	9.6±0.776.5±0.34	–	25.9±3.4912±1.39

BL= Bilobate, Cl= Clavate, CR= Cross, DT= Dendritic, LCN= Lenceolate, OVL= Oval, RD= Rondel, RT= Rectangular, SD=saddle, SmE= Smooth elongate, TZ= Trapezoid. *=Mean Length ± Standard error, #=Mean breadth± Standard error

Table 2: Common Names, Ecology and Distribution of Grasses from Punjab Used for Present Study

Name of Species	Common name (s)	Subfamily	Ecology and Distribution
Cynodon dactylon **(L.) Pers**	Bermuda Grass	Chloridoideae	Present throughout India.
Tragus biflorus **Schult.**	Sandbur Grass	Chloridoideae	Xerophytic areas of Punjab plains
Dactyloctenium aegyptium **(L.) Willd.**	Egyptian Crowfoot Grass	Chloridoideae	Waste lands in tropical regions of India
Desmostachya bipinnata **(L.) Stapf**	Cord Grass/ Salt Reed Grass	Chloridoideae	Grows throughout India.
Eleusine indica **(L.) Gaertn.**	Crowsfoot Grass/Goose Grass	Chloridoideae	Tropical and Subtropical Regions of India.
Leptochloa chinensis **(L.) Nees**	Red SprangleTop	Chloridoideae	Waste regions of South East India
Leptochloa panicea **(Retz) Ohwi**.	Mucronate Sprangle Top	Chloridoideae	Waste lands of South East India and Punjab
Neyraudia arundinacea (**L.**) **Henrard**	Madagascar Grass	Chloridoideae	Common in Shiwalik hills of Punjab
Sporobolus diandrus **(Retz.) P. Beauv**	Tussock dropseed Grass	Chloridoideae	Tropical to Warm Temperate regions

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